

Free Amino Acids and Derivatives in Eggs of *Tenebrio molitor* During Development

DONALD S. PO-CHEDLEY

D'YOUVILLE COLLEGE, BUFFALO, N.Y. 14201

RECEIVED FOR PUBLICATION NOVEMBER 27, 1968

Abstract: Seventeen free amino acids and three derivatives, asparagine, glutamine, and taurine, were identified in the developing eggs of the meal worm and have been quantitatively determined. Changes in concentration for these compounds showed an increase in total concentration by the fifth day of development, and a decrease in volume to the last day, the eighth, which, nevertheless, was higher than the concentration at the start of development.

Although insects are characterized by having high concentrations of free amino acids, there is no definite agreement as to the roles they play in insect metabolism (Roeder, 1953). The available literature pertains, mostly, to post-embryonic stages and shows that the common amino acids and the amides, asparagine, glutamine, and taurine, are present (Gilmour, 1961; Chen, 1962). Similar reports for the developing egg are rather sparse. The general consensus indicates that the amino acid pool in the developing egg is comparable to the adult (Chen, 1966).

Recent investigations assigning a physiological significance to the high titre of free amino acids and their fluctuations during development have been noted by Henry and Cook (1963) for the black blow fly, *Phormia regina*, and by Pant and Agrawal (1965) for the moth, *Philosamia ricini*. Metabolic responses associated with the break down of pre-existing yolk reserves and leading to the formation of tissue and organ specific proteins through free amino acid and peptide intermediates have been studied by Colombo (1962) and Chen (1967).

The present study provides data showing amino acid patterns existing for each day of the developing meal worm embryo. Studies of the amino acid history during embryogenesis will provide data necessary for interpreting the accompanying morphological and physiological events.

MATERIAL AND METHODS

Newly emergent adults were collected daily from a pupal reservoir and transferred to a bread-meal culture for a five day conditioning period. During this time they developed their black color. These adults were then placed in a glass specimen dish containing flour for the subsequent egg collections. The eggs were harvested daily by sifting the flour through a 0.45 mm. mesh sieve, sorted

Editor's Note: Word has been received that Dr. Po-Chedley died on April 1, 1969.

according to age, stored in separate vials and incubated at 25°C., 70 per cent R.H. Under these conditions the embryological period is eight days.

The techniques of paper and thin layer chromatography were used for amino acid determinations. Fifty milligrams of eggs (about 100 eggs) were homogenized in a 15 ml. centrifuge tube and extracted with 10 ml. of 95 per cent ethanol for 30 minutes. The homogenate was centrifuged and the clear supernatant fluid collected. The residue was re-extracted with 5 ml. of 95 per cent ethanol until the supernate was negative to ninhydrin. The pooled supernates were evaporated down to a 1 ml. volume for chromatographic determinations. One to five microliters of the pooled sample were used for both one- and two-dimensional runs. The chromatogram sheets were sprayed with 0.5 per cent ninhydrin, and dried at 60°C. for 15 minutes for the development of the amino acid colors. Besides ninhydrin, other spray reagents were used to identify specific amino acids, arginine, proline, tyrosine and sulphur compounds (Pant, 1965). Quantitative determinations were made by cutting the paper into strips and measuring color intensity of individual spots by direct photometry.

OBSERVATIONS

A total of twenty free ninhydrin positive compounds were identified on all the chromatograms and their concentrations recorded for the eight days of meal worm embryogenesis (Table I). The daily changes in total concentrations for the amino acids show a diphasis response. The concentrations range from 156.7 to 196.3 mg. per cent from the first to the fifth day of growth. The values subsequently decrease to 166.7 mg. per cent by the eighth day. The high concentrations existing at the fourth and fifth days of embryology occur at the time when larval morphology has become established. This interval serves as a useful criterion for evaluating amino acid relationships to metabolic processes which provide some insight into morphological and physiological events.

The behaviour of certain amino acids and derivatives may be noted. Glycine, the most abundant amino acid, decreased steadily in concentration during the first five days of growth. Tyrosine and phenylalanine show opposing changes in concentration in that the tyrosine history is U-shaped and phenylalanine is Ω -shaped.

During the decrease of glutamic and aspartic acids for the eight days of growth there was a steady increase in the concentration of their amides, glutamine, and asparagine. Both the sulphur amino acids, cystine and methionine, increased steadily during the period of growth. The compound in lowest concentration during the entire period was taurine.

DISCUSSION

The seventeen free amino acids and three derivatives found in meal worm eggs were present in all the analysed stages. The change in total concentration during

TABLE I. Changes in ninhydrin positive compounds determined in the developing meal worm embryo; values expressed in mg. per cent amino nitrogen.

compounds	days of growth							
	1	2	3	4	5	6	7	8
alanine	8.5	9.2	8.4	12.6	14.2	15.1	13.2	13.1
arginine	4.8	4.6	5.0	5.6	5.1	4.3	4.7	3.0
asparagine	2.5	3.0	5.2	6.0	6.2	6.8	6.6	11.2
aspartic acid	14.2	14.7	14.0	12.1	10.5	10.1	9.8	3.1
cystine	6.5	5.5	5.8	7.2	8.0	10.0	13.2	14.5
glutamic acid	15.5	16.0	12.0	11.2	12.0	8.6	4.2	2.1
glutamine	3.3	6.1	9.2	11.6	12.2	13.1	11.0	13.2
glycine	20.5	18.6	17.1	15.1	13.2	14.9	15.0	15.0
histidine	5.1	7.6	8.2	10.1	8.7	7.9	7.0	4.9
leucines	12.2	11.5	11.9	12.9	12.1	12.7	13.8	13.8
lysine	8.0	9.1	9.2	10.1	10.4	9.8	9.2	8.6
methionine	4.6	6.2	8.3	8.4	8.4	7.5	9.1	9.2
phenylalanine	12.2	14.9	16.1	18.1	18.0	17.2	15.1	12.2
proline	8.2	10.2	13.2	15.7	16.4	13.9	12.1	9.2
serine	4.6	5.0	5.9	7.2	7.0	6.8	6.2	6.1
taurine	2.5	1.8	1.4	1.6	1.7	2.0	1.8	1.8
threonine	3.5	4.0	5.1	6.2	6.0	4.1	3.0	3.0
tryptophane	5.7	4.3	6.1	7.9	7.0	7.0	7.8	6.9
tyrosine	9.5	9.1	5.9	3.6	3.0	2.2	5.2	9.7
valine	4.8	5.1	5.9	7.9	6.2	6.7	6.5	6.1
totals	156.7	166.6	173.9	189.5	196.3	180.7	170.6	166.7

the course of embryogenesis for the meal worm has been similarly noted for the locust by Colombo (1962) and for the mosquito by Chen and Briegel (1965). It was suggested (Colombo, 1962) that such concentration changes were due to mobilization of yolk reserves for protein synthesis during the first phase of embryogenesis. During the latter part of development the decrease in amino acid concentration indicated a decline in yolk proteolysis. The amino acid pool satisfied the requirements for differentiation as the final amino acid concentrations are higher than those noted at the start of development.

An examination of changes in concentration for individual amino acids may account for certain aspects of metabolic activity prevailing during the course of development.

Glycine was present in highest concentration at the start of development. It may be that this initial effect was related to the reservoir of glycine in the yolk of the one day old egg (Colombo, 1962). The gradual increase in concentration for glycine at the end of development appears to substantiate Kutscher and Ackermann's (1933) contention that the velocity of insect metabolism prevented the acid from accumulating in high concentration.

The initially high aggregates of aspartic and glutamic acids are followed by a steady decrease in volume while the corresponding amides, asparagine, and glutamine, increase in concentration during the same period of growth. These

reactions indicate an interconversion of these compounds (Kilby and Neville, 1957; Colombo, 1962; Chen, 1966).

Published data (Chen and Briegel, 1965) shows a clear correlation between tyrosine concentrations and pigmentation development. The *Tenebrio* embryo, however, shows no discernible pigmentation during its period of growth. Pigmentation occurs in the larva about three days following its eclosion. In this instance the high concentration of tyrosine accumulated at the end of embryology would act as the reserve necessary for larval pigmentation. The early decrease of tyrosine and the corresponding rise of phenylalanine and cystine occur at the time, the third through the fifth day of growth, when cuticular synthesis occurs (Golberg and DeMeillon, 1948; Colombo, 1962).

Both sulphur containing amino acids, cystine and methionine, increased in volume during the growth period. In *Melanoplus differentialis* embryos a transfer of sulphur between these compounds was reported by Fu (1957). The impression that cystine acts as a precursor for taurine is not demonstrated in the meal worm data. The constant volume of taurine and increasing concentration of cystine during the period of growth rules out such a possibility at this time.

Although the enumeration of the amino acids and derivatives present during meal worm embryogenesis does not explain physiological changes, it does provide a framework within which examination of individual compounds may be furthered. The functions of the different compounds may be more fully interpreted when different tissues are analysed (Deuchar, 1958) and when yolk and embryos are separately examined (Colombo, 1962).

Literature Cited

- CHEN, P. S. 1962. Free amino acids in insects. In "amino acid pools." p. 115-138, J. T. Holden, Elsevier, Amsterdam.
- . 1966. Amino acid and protein metabolism in insect development. *Adv. Insect Physiol.*, **3**: 53-132.
- AND H. BRIEGEL. 1965. Studies on the protein metabolism of *Culex pipiens* L. Changes in free amino acids and peptides during embryonic development. *Comp. Biochem. Physiol.*, **14**: 463-473.
- , F. HANIMANN AND H. BRIEGEL. 1967. Freie aminosäuren und derivate in eiern von *Drosophila*, *Culex* und *Phormia*. *Revue. Suisse de Zool.*, **74**: 570-589.
- COLOMBO, G., C. A. BENASSI, G. ALLEGRI AND E. LONGO. 1962. Free amino acids in eggs of *Schistocerca gregaria* Forsk. (Orthoptera) during development. *Comp. Biochem. Physiol.*, **5**: 83-93.
- DEUCHAR, E. M. 1956. Amino acids in developing tissue of *Xenopus laevis*. *J. Embryol. Exp. Morph.*, **4**: 327-346.
- FU, Y. Y. 1957. Changes in distribution of sulphur-containing amino acids in developing grasshopper eggs (*Melanoplus differentialis*). *Physiol. Zool.*, **30**: 1-12.
- GILMOUR, D. 1961. The biochemistry of insects. Chapter IX. Academic Press, New York.
- GOLBERG, L. AND B. DEMEILLON. 1948. The nutrition of the larvae of *Aedes aegypti* Linnaeus. *Biochem. J.*, **43**: 379-387.
- HENRY, M. J. AND T. W. COOK. 1963. Amino acids in protein of the black blow fly,

- Phormia regina* (Meigen), at various stages of development. Contrib. Boyce Thompson Instit., **22**: 133-140.
- KILBY, B. A. AND E. NEVILLE. 1957. Amino acid metabolism in locust tissues. J. Exp. Biol., **34**: 276-289.
- KUTSCHER, F. AND D. ACKERMANN. 1933. Comparative biochemistry of vertebrates and invertebrates. Ann. Rev. Biochem., **2**: 365-376.
- PANT, R. AND H. C. AGRAWAL. 1965. Amino acid metabolism during embryonic development of *Philosamia ricinis* (Lepidoptera). J. Ins. Physiol., **11**: 387-389.
- ROEDER, K. D. 1953. Insect Physiology. p. 174. J. Wiley and Sons, New York.
- YOSHITAKE, N., H. ARUGA AND Y. SUZUKI. 1950. Studies of the amino acids in the silk worm. I. On the free amino acids in the silk worm eggs. J. Seric. Sci. Tokyo, **19**: 530-535.